ORIGINAL ARTICLE

UDC: 616.13-004.6-02
DOI: 10.2298/VSP140417074T

Copper and zinc concentrations in atherosclerotic plaque and serum in relation to lipid metabolism in patients with carotid atherosclerosis

Koncentracija bakra i cinka u aterosklerotskom plaku i serumu u odnosu na metabolizam lipida kod bolesnika sa karotidnom aterosklerozom

Nebojša M. Tasić*, Danijela Tasić*, Petar Otašević*, Mirjana Veselinović†, Vladimir Jakovljević‡, Dragan Djurić§, Djordje Radak*

***Dedinje Cardiovascular Institute, §Institute of Physiology "Richard Burian", Faculty of Medicine, University of Belgrade, Belgrade, Serbia; †Department of Rheumatology, Internal Medicine Clinic, Clinical Centre Kragujevac, ‡Department of Physiology, Faculty of Medicine, University of Kragujevac, Kragujevac, Serbia**

Abstract

Background/Aim. Some oligoelements are now investigated as possibly having a role in atherosclerosis. The aim of this study was to compare the concentrations of copper and zinc in the serum and carotid plaque and parameters of lipid metabolism in patients with different morphology of carotid atherosclerotic plaque. **Methods.** Carotid endarterectomy due to the significant atherosclerotic stenosis was performed in 91 patients (mean age 64 ± 7). The control group consisted of 27 patients (mean age 58 ± 9), without carotid atherosclerosis. Atherosclerotic plaques were divided into four morphological groups, according to ultrasonic and intraoperative characteristics. Copper and zinc concentrations in the plaque, carotid artery and serum were measured by atomic absorption spectrophotometry. **Results.** Serum copper concentrations were statistically significantly higher in the patients with hemorrhagic in comparison to those with calcified plaque (1.2 ± 0.9 μmol/L *vs* 0.7 ± 0.2 μmol/L, respectively; p = 0.021). Zinc concentrations were statistically significantly lower in plaques of the patients with fibrolipid in comparison to those with calcified plaques (22.1 ± 16.3 μg/g *vs* 38.4 ± 25.8 μg/g, respectively; p = 0.024). A negative significant correlation was found for zinc and triglycerides in the serum in all the patients (r = -0.52, p = 0.025). In the control group we also demonstrated a positive significant correlation for low-density lipoprotein cholesterol and copper in the serum (r = 0.54, p = 0.04). **Conclusion.** The data obtained in the current study are consistent with the hypothesis that high copper and lower zinc levels may contribute to atherosclerosis and its sequelae as factors in a multifactorial disease. Further studies are necessary in order to conclude whether high concentration of copper and zinc in the serum could be risk factors for atherosclesrosis.

Key words:
zinc; copper; risk factors; arteriosclerosis; carotid stenosis; lipids.

Apstrakt

Uvod/Cilj. Neki oligoelementi sada se istražuju zbog moguće uloge u aterosklerozi. Cilj ovog istraživanja bio je da se uporede koncentracije bakra i cinka u serumu i karotidnom aterosklerotskom plaku i parametri metabolizma lipida kod bolesnika sa različitim morfologijama karotidnog plaka. **Metode.** Karotidna endarterektomija zbog klinički napredovale ateroskleroze urađena je kod 91 bolesnika starosti 64 ± 7 godina. Kontrolnu grupu činilo je 27 bolesnika prosečne starosti 58 ± 9 godina, bez prisutne karotidne ateroskleroze. Prema morfologiji, aterosklerotski plakovi su podeljeni u četiri grupe na osnovu ultrazvučnog nalaza i intraoperativnih karakteristika. Spektrofotometrijski su određivane koncentracije bakra i cinka u aterosklerotskom plaku, zidu karotidne arterije i serumu. **Rezultati.** Koncentracija bakra u serumu bila je statistički značajno viša kod bolesnika koji su imali hemoragičan plak u odnosu na bolesnike sa kalcifikovanim karotidnim plakom (1,2 mL ± 0.9 *vs* 0,7mL ± 0,2; p = 0,021). Kod bolesnika sa fibrolipidnim plakom koncentracija cinka u samom plaku bila je statistički značajno niža u odnosu na koncentraciju cinka u kalcifikovanom karotidnom plaku (22,1 ± 16,3 μg/g *vs* 38,4 ± 25,8 μg/g; p = 0,024). Kod svih bolesnika u studiji pronađena je negativna korelacija između serumske koncentracije cinka i vrednosti triglicerida u serumu (r = -0.52, p = 0.025). U kontrolnoj grupi serumska koncentracija bakra bila je u pozitivnoj korelaciji sa lipoproteinima holesterola male gustine (r = 0,54, p = 0,04). **Zaključak.** Rezultati dobijeni u ovoj studiji podudaraju se sa pretpostavkom da visoke koncentracije bakra i cinka u serumu mogu doprineti razvoju ateroskleroze i njenih kliničkih manifestacija. Potrebna su dalja klinička istraživanja koja bi dokazala da visoke koncetracije bakra i cinka u serumu mogu biti faktori rizika od nastanka i razvoja ateroskleroze.

Ključne reči:
cink; bakar; faktori rizika; arterioskleroza; aa. carotis, stenoza; lipidi.

Correspondence to: Nebojša M. Tasić, "Dr Aleksandar D. Popović" Cardiovascular Research Center, Dedinje Cardiovascular Institute, Milana Tepića 1, 11 040 Belgrade, Serbia. Phone: +381 11 3601 669, Fax: +381 11 2666 445. E-mail: nebtsa@yahoo.com

Introduction

The positive correlation of elevated low-density lipoprotein (LDL) cholesterol serum levels and inverse correlation of high-density lipoprotein (HDL) cholesterol levels with the atherosclerotic process is a mainstay for research activities regarding pathophysiology and treatment of atherosclerotic clinical manifestation [1].

In recent years investigators have focused their research activities on the role of several trace elements in the pathogenesis of atherosclerosis. Among trace elements, copper and zinc are well-documented as important modulating factors in cardiovascular homeostasis. Special research interest has been given to the relationship between copper and zinc serum and tissue concentrations and lipid status. Several studies pointed out a significant role of copper in LDL oxidation, however the exact mechanism is not yet understood [2]. Copper has the pro- and anti-atherogenic (dual) effect [3]. Some experimental studies showed a positive correlation of copper deficiency with high total cholesterol levels and atherosclerotic changes on coronary arteries [4]. Low copper intake with copper defficiency and high dietary zinc/copper ratio leads to the increase in total serum cholesterol and development of atherosclerotic coronary disease [5]. Other studies implied that high copper serum levels could lead to the endothelial damage and the beginning of the atherosclerotic process [6].

Zinc is an essential trace element for the normal membrane structure and function of important enzymes [7]. Studies reported that low zinc concentration may have an important role in the pathogenesis of atherosclerosis [8]. The effect of high zinc concentration on endothelial cells is not yet understood. Some evidence suggest that zinc can act as an endogenous protective factor against atherosclerosis by inhibiting the oxidation of LDL by cells or transition metals and protect the cell against cell-destabilizing agents such as inflammatory cytokines and polyunsaturated lipids [9].

Through the interaction with endogenic basic fibroblast growth factor zinc potentiates endothelial and vascular smooth muscle cell proliferation which is an important part of intimal hyperplasia during the atherosclerotic process [10].

Diversity and heterogeneity of atherosclerotic plaques implies that the atherosclerotic process is dynamic, complex and continuous. Numerous factors influence the morphology and growth pattern of different atherosclerotic plaques.

The aim of this study was to compare the concentrations of copper and zinc in the serum and carotid plaque and parameters of lipid metabolism in patients with different morphology of carotid atherosclerotic plaques.

Methods

The study group consisted of 118 subsequent patients (mean age 63 ± 8 years; 54.2% males) admitted to the Department of Vascular Surgery at Dedinje Cardiovascular Institute in Belgrade for carotid surgery. Carotid endarterectomy due to significant atherosclerotic stenosis and symptoms of cerebrovascular insufficiency was performed in 91 patients (mean age 64.2 ± 6.9 years, 62.6% males). The control group consisted of 27 patients (mean age 58.4 ± 9.3 years, 25.9% males) without carotid atherosclerosis which were operated due to the symptomatic kinking and coiling of carotid artery.

All the patients underwent neurological, cardiological and vascular surgeon exam, carotid artery ultrasound measurements and were treated surgically. Blood samples were drawn from all the patients before the surgery and sera were analyzed for the concentrations of lipid parameters (total cholesterol, HDL- and LDL-cholesterol and triglycerides), copper and zinc.

Tissue samples, atherosclerotic plaques from the atherosclerotic patients and normal carotid tissue from the control group patients were analyzed for the concentrations of copper and zinc. In the atherosclerotic subjects, atherosclerotic plaques were divided into four morphological groups, namely fibrolipid, hemorrhagic, ulcerated, and calcified plaque, according to ultrasonic and intraoperative characteristics [11].

The study was approved by the Ethics Committee of the Dedinje Cardiovascular Institute. Written consent was obtained from all the patients before they entered the study.

Total cholesterol concentration in the serum was determined by the reaction with cholesterol-oxidase by using a diagnostic kit by Abbott. HDL-cholesterol in the serum was determined with the direct method by using a diagnostic kit by Abbott. Triglycerides in the serum were estimated by the reaction with glycerol-oxidase by using a diagnostic kit Abbott. LDL cholesterol was calculated according to the obtained levels of other lipid parameters noted above.

Copper and zinc concentrations in plaques, carotid artery tissue and the serum were estimated by means of flame atomic absorption spectrophotometry (AAS) using the Varian AA-5 instrument under the conditions recommended by the producer (acetylene/air flame, wavelenght: $\lambda Cu = 324.75$ nm, $\lambda Zn = 213.86$ nm).

Data are presented graphically as a box-plots, showing the mediana, minimum and maximum values. Differences in quantitative variables were assessed using the Mann-Whitney *U* test. The associations between the groups were analyzed by the Spearman's correlation. A p-value < 0.05 was considered statistically significant. Data were analyzed by SPSS version 10 (SPSS Inc, Chicago, Illinois, USA).

Results

Our patients cohort exhibited most of the classic atherosclerosis risk factors (Table 1). The percentage of hypertensive, smoking subjects was higher in the patients with carotid plaque than in the controls ($p < 0.05$). The presence of diabetes mellitus showed statistically significant differences between the patients and the controls ($p < 0.05$).

Atherosclerotic subjects were divided into four groups according to different morphology of analyzed atherosclerotic plaques [11]. The group I (22 patients; 18.6%) represented the patients with fibrolipid atherosclerotic plaques. The group II included the patients with hemorrhagic plaques (8 patients; 6.8%), the group III included the patients with ul-

Table 1

Baseline characteristics of the patients with carotid atherosclerosis (the study group) and the control group of patients

Variable	Study group (n = 91)	Control group (n = 27)	*p* values
Age (years), $\bar{x} \pm SD$	64.2 ± 6.9	58.4 ± 9.3	$p > 0.05$
Hypertension	90.1	59.3	$p < 0.05$
Smokers	67	40.7	$p < 0.05$
Diabetes mellitus	26.4	14.8	$p > 0.05$
Famiily history	49.4	55.6	$p > 0.05$
Carotid artery bruit	90.1	33.3	$p < 0.05$

Results are given as percentage of patients

cerated plaques (31 patients; 26.3%), and the group IV included the patients with calcified atherosclerotic plaques (30 patients; 25.4%). The patients from the control group, without significant atherosclerotic changes in carotid artery, constituted the group V (27 patients; 22.9%).

Values of the distribution of HDL- and LDL-cholesterol between the different plaques morphology groups and controls are presented in Figures 1 and 2, respectively.

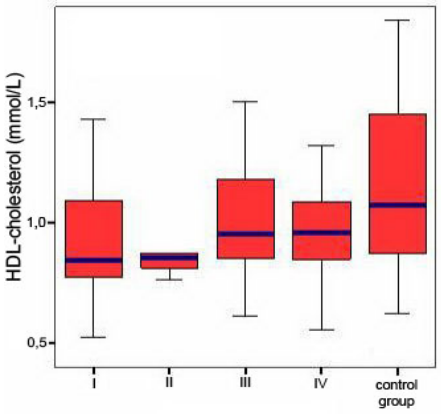

Fig. 1 – Average high-density lipoprotein (HDL) cholesterol values in patients with different morphological groups of atherosclerotic plaques. Groups of patients: I – with fibrolipid atherosclerotic plaque; II – with hemorrhagic plaque; III – with ulcerated atherosclerotic plaque; IV – with calcified atherosclerotic plaque.

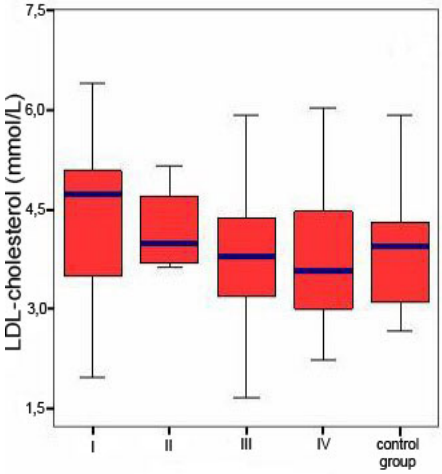

Fig. 2 – Average low-density lipoprotein (LDL) cholesterol values in patients with different morphological groups of atherosclerotic plaques (for explanation see under Figure 1)

The distribution of copper concentration in plaque, carotid tissue and the serum are shown in Figures 3 and 4, respectively. Our study revealed a statistically significantly higher average serum copper concentration in the patients with hemorrhagic plaque (the group II) compared to the patients with calcified (the group IV) atherosclerotic plaques (1.2 μg/g ± 0.9 *vs* 0.7 μg/g ± 0.2, respectively; $p < 0.05$). The average values of copper concentrations in morphologically different atherosclerotic plaques and normal carotid tissue were similar.

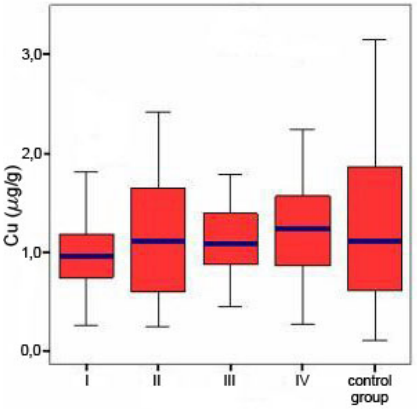

Fig. 3 – Average plaque copper concentration in the different groups of patients (for explanation see under Figure 1).

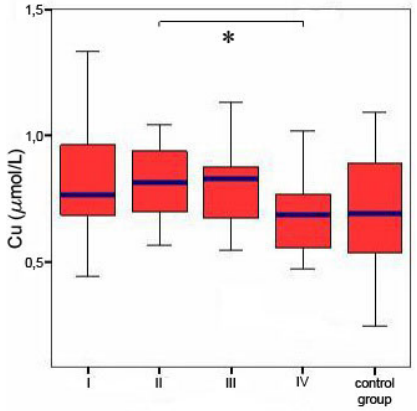

Fig. 4 – Average serum copper concentration in the different groups of patients.

***p < 0.05 (statistically significant difference between the groups II and IV). (for explanation see under Figure 1)**

No significant difference in the serum zinc concentrations was found among the groups (Figure 5). We found a statistically significantly lower average zinc plaque concen-

tration in the patients with fibrolipid plaque (the group I) in comparison to the patients with calcified (the group IV) plaques (22.1 ± 16.3 μg/g *vs* 38.4 ± 25.8 μg/g respectively; $p = 0.024$), (Figure 6).

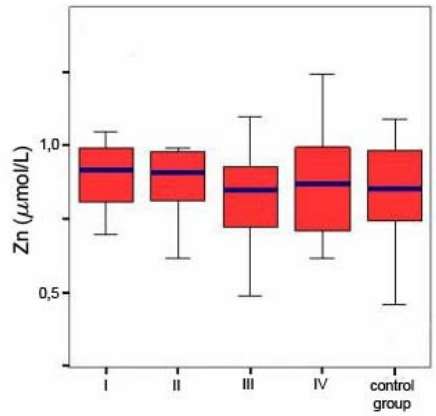

Fig. 5 – Average serum zinc concentration in the different groups of patients (for explanation see under Figure 1).

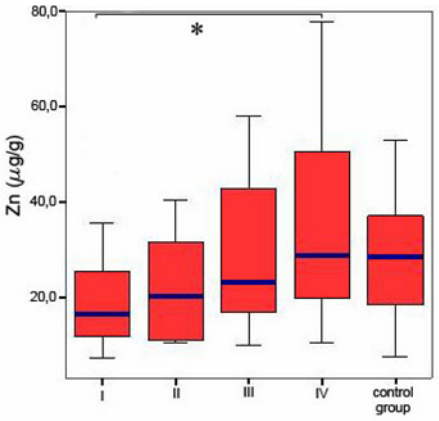

Fig. 6 – Average plaque zinc concentration in the different groups of patients.

***p < 0.05 (statistically significant difference between the groups II and IV) (for explanation see under Figure 1)**

The negative and significant correlation coefficients were calculated for zinc and triglycerides in the selected patients ($r = 0.52$, $p = 0.025$) (data not showed).

In the control group we also demonstrated positive significant correlation coefficient for LDL-cholesterol and copper in the serum ($r = 0.54$, $p = 0.04$) (data not showed).

Discussion

Metal ions have been proposed as causative agents in a number of diseases, including atherosclerosis. The involvement of transition metals in atherosclerosis is controversial. Experimental studies have reported elevated levels of iron and copper in diseased human arteries but have often used methods that release metal ions from proteins [12]. Elevated levels of copper were also detected in carotid lesions [13]. Other studies do not support the hypothesis that elevated metal ion levels may be a major causative factor in aggravated atherosclerosis [14].

Copper can promote low-density lipoprotein oxidation and the formation of macrophage-derived foam (lipid-laden) cells *in vitro* [15]. These elevated metal ion levels may therefore affect plaque stability and propensity to rupture.

Our study revealed a significantly higher copper concentration in the serum of patients with complicated hemorrhagic plaque in comparison to the morphological group IV (calcified plaque). There are no consistent data in the literature regarding the relationship between serum copper concentrations and the severity of atherosclerotic process [16].

An Iranian study revealed a significantly higher serum copper concentration in patients with coronary heart disease of both sexes compared to controls [17].

Diaz Romero et al. [18] determined equivalent serum copper values in patients with coronary heart disease and controls. Correspondingly to our results Alissa et al. [19] revealed no significant difference in both copper and zinc serum concentrations between the patients with established atherosclerosis and controls.

Our investigation showed a similar average copper concentration in atherosclerotic plaques and normal carotid tissue. The previous study of our group reported significantly higher copper concentrations in patients with ulcerated plaque in comparison to copper concentration in normal carotid tissue [20]. Serum copper concentrations are also associated with other coronary risk factors, including body mass index, levels of physical activity, serum HDL-cholesterol and C-reactive protein (CRP) [21].

Polish authors [22] in their investigations demonstrated higher copper concentration in atherosclerotic plaques of patients with peripheral arterial disease in comparison to controls.

Our results show a trend of higher serum zinc concentration in the patients with fibrolipid and hemorrhagic plaques than in the patients in other morphology groups of atherosclerotic plaques. We found no significant difference between serum zinc levels in atherosclerotic and normal subjects. We found a significantly lower average zinc plaque concentration in the patients with fibrolipid plaques in comparison to the patients with calcified plaques. Stadler et al. [23] found elevated levels of zinc in human atherosclerotic advanced lesions carotid and abdominal artery compared to healthy tissue or early lesions. The control subjects had no significantly higher plaque zinc levels compared to noncalcified atherosclerotic plaque patients which is in accordance with our earlier investigation [20].

Recent studies reported lower zinc serum levels in patients with different clinical form of atherosclerosis compared to controls and suggested that protective effect of zinc accumulation in atherosclerotic plaque could be associated with lesion calcification [23, 24]. Patients with the established coronary artery disease had significantly higher serum CRP and lower serum zinc compared to both patients without coronary artery disease and healthy controls [16]. Zinc deficiency has been associated with the development of atherosclerosis [23].

The presented higher proportion of male subjects in atherosclerotic groups and female subject in patients with

kinking and coiling carotid artery is in accordance with the results of earlier studies [25]. The atherosclerotic subjects in our study had higher LDL cholesterol and lower HDL cholesterol serum levels in comparison to the control subjects. Previous studies also demonstrated a significant positive correlation of LDL values and negative correlation of HDL values with the severity of the carotid atherosclerotic process [26]. The investigators from the "Tromso study" reported a strong positive correlation of HDL values and reduction of atherosclerotic plaque progression, higher plaque echogenity and in some cases a reduction in plaque size [27].

We found a significant negative correlation between serum zinc concentration and triglycerides. Serum copper concentrations were positively associated with fasting serum triglycerides in Iranian patients [16]. In the control group we demonstrated a positive significant correlation of LDL cholesterol and copper concentration in the serum.

Human studies investigating the effect of copper and zinc status on cholesterol metabolism are very limited [28]. In an adult African-American community females taking either zinc supplements had higher HDL-cholesterol values than nonsupplementing females [29].

Serum zinc and copper levels were both significantly lower in individuals with normal versus high levels of LDL-cholesterol in a large Iranian population sample [30]. One of the theory implies that hypercholesterolemia and endothelial damage lead to peroxynitrite formation, decrease in pH and increase in copper ion release from ceruloplasmin. This process enhance LDL oxidation and plaque growth. Several studies failed to confirm a significant correlation between oligoelements and lipoprotein fractions [28]. Iranian authors [17] reported no significant relationship between copper and zinc serum concentration and different lipoprotein fractions in atherosclerotic patients.

Conclusion

Overall, the data obtained in the current study are consistent with the hypothesis that copper and zinc may contribute to atherosclerosis and its sequelae as factors in a multifactorial disease. Registered differences in oligoelements between different morphological groups should be further evaluated in clinical settings of different oligoelements intake.

REFERENCES

1. *Calabresi L, Gomaraschi M, Simonelli S, Bernini F, Franceschini G.* HDL and atherosclerosis: Insights from inherited HDL disorders. Biochim Biophys Acta 2014; pii: S1388−1981(14)00148-6.
2. *Horsley ET, Burkitt MJ, Jones CM, Patterson RA, Harris LK, Moss NJ,* et al. Mechanism of the antioxidant to pro-oxidant switch in the behavior of dehydroascorbate during LDL oxidation by copper(II) ions. Arch Biochem Biophys 2007; 465(2): 303−14.
3. *Rajendran R, Ren M, Ning P, Tan KH, Halliwell B, Watt F.* Promotion of atherogenesis by copper or iron--which is more likely. Biochem Biophys Res Commun 2007; 353(1): 6−10.
4. *Hamilton IM, Gilmore WS, Strain JJ.* Marginal copper deficiency and atherosclerosis. Biol Trace Elem Res 2000; 78(1−3): 179−89.
5. *Ghayour-Mobarhan M, Taylor A, Kazemi-Bajestani SM, Lanham-New S, Lamb DJ, Vaidya N,* et al. Serum zinc and copper status in dyslipidaemic patients with and without established coronary artery disease. Clin Lab 2008; 54(9−10): 321−9.
6. *Lamb DJ, Avades TY, Ferns GA.* Biphasic modulation of atherosclerosis induced by graded dietary copper supplementation in the cholesterol-fed rabbit. Int J Exp Pathol 2001; 82(5): 287−94.
7. *Mccord MC, Aizenman E.* The role of intracellular zinc release in aging, oxidative stress, and Alzheimer's disease. Front Aging Neurosci 2014; 6: 77. .
8. *Prasad AS.* Zinc: role in immunity, oxidative stress and chronic inflammation. Curr Opin Clin Nutr Metab Care 2009; 12(6): 646−52.
9. *Leone N, Courbon D, Ducimetiere P, Zureik M.* Zinc, copper, and magnesium and risks for all-cause, cancer, and cardiovascular mortality. Epidemiology 2006; 17(3): 308−14.
10. *Maret W.* Molecular aspects of human cellular zinc homeostasis: redox control of zinc potentials and zinc signals. Biometals 2009; 22(1): 149−57.
11. *Beletsky VY, Kelley RE, Fowler M, Phifer T.* Ultrasound densitometric analysis of carotid plaque composition. Pathoanatomic correlation. Stroke 1996; 27(12): 2173−7.
12. *Stadler N, Heeneman S, Vöö S, Stanley N, Giles GI, Gang BP,* et al. Reduced metal ion concentrations in atherosclerotic plaques from subjects with type 2 diabetes mellitus. Atherosclerosis 2012; 222(2): 512−8.
13. *Stadler N, Lindner RA, Davies MJ.* Direct detection and quantification of transition metal ions in human atherosclerotic plaques: evidence for the presence of elevated levels of iron and copper. Arterioscler Thromb Vasc Biol 2004; 24(5): 949−54.
14. *Islamoglu Y, Evliyaoglu O, Tekbas E, Cil H, Elbey MA, Atilgan Z,* et al. The relationship between serum levels of Zn and Cu and severity of coronary atherosclerosis. Biol Trace Elem Res 2011; 144(1−3): 436−44.
15. *Burkitt MJ.* A critical overview of the chemistry of copper-dependent low density lipoprotein oxidation: roles of lipid hydroperoxides, alpha-tocopherol, thiols, and ceruloplasmin. Arch Biochem Biophys 2001; 394(1): 117−35.
16. *Kazemi-Bajestani SM, Ghayour-Mobarhan M, Ebrahimi M, Moohebati M, Esmaeili HA, Parizadeh MR,* et al. Serum copper and zinc concentrations are lower in Iranian patients with angiographically defined coronary artery disease than in subjects with a normal angiogram. J Trace Elem Med Biol 2007; 21(1): 22−8.
17. *Nourmohammadi I, Nazem N, Ehsani-Zenuz A, Moaveni A.* Serum levels of Zn, Cu, Cr and Ni in Iranian subjects with atherosclerosis. Arch Irn Med 2001; 4(1): 21−4.
18. *Díaz Romero C, Henríquez Sanchez P, López Blanco F, Rodríguez Rodríguez E, Serra Majem L.* Serum copper and zinc concentrations in a representative sample of the Canarian population. J Trace Elem Med Biol 2002; 16(2): 75−81.
19. *Alissa EM, Bahjri SM, Ahmed WH, Al-Ama N, Ferns GA.* Trace element status in Saudi patients with established atherosclerosis. J Trace Elem Med Biol 2006; 20(2): 105−14.
20. *Radak D, Cvetković Z, Tasić N, Petrović B, Lacković V, Djordjević-Denić G.* The content of copper and zinc in human ulcerated carotid plaque. Srp Arh Celok Lek 2004; 132(3−4): 80−4. (Serbian)

21. *Ghayour-Mobarhan M, Taylor A, New SA, Lamb DJ, Ferns GA.* Determinants of serum copper, zinc and selenium in healthy subjects. Ann Clin Biochem 2005; 42(Pt 5): 364−75.
22. *Iskra M, Majewski W, Piorunska-Stolzmann M.* Modifications of magnesium and copper concentrations in serum and arterial wall of patients with vascular diseases related to aging, atherosclerosis and aortic aneurysm. Magnes Res 2002; 15(3−4): 279−85.
23. *Stadler N, Stanley N, Heeneman S, Vacata V, Daemen MJ, Bannon PG,* et al.. Accumulation of zinc in human atherosclerotic lesions correlates with calcium levels but does not protect against protein oxidation. Arterioscler Thromb Vasc Biol 2008; 28(5): 1024−30.
24. *Allen-Redpath K, Ou O, Beattie JH, Kwun IS, Feldmann J, Nixon GF.* Marginal dietary zinc deficiency in vivo induces vascular smooth muscle cell apoptosis in large arteries. Cardiovasc Res 2013; 99(3): 525−34.
25. *Togay-Isikay C, Kim J, Betterman K, Andrews C, Meads D, Tesh P,* et al. Carotid artery tortuosity, kinking, coiling: stroke risk factor, marker, or curiosity?. Acta Neurol Belg 2005; 105(2): 68−72.
26. *Kawamoto R, Oka Y, Tomita H, Kodama A.* Non-HDL cholesterol as a predictor of carotid atherosclerosis in the elderly. J Atheroscler Thromb 2005; 12(3): 143−8.
27. *Johnsen SH, Mathiesen EB, Fosse E, Joakimsen O, Stensland-Bugge E, Njølstad I,* et al. Elevated high-density lipoprotein cholesterol levels are protective against plaque progression: a follow-up study of 1952 persons with carotid atherosclerosis the Tromsø study. Circulation 2005; 112(4): 498−504.
28. *Lopes PA, Santos MC, Vicente L, Rodrigues MO, Pavão ML, Nève J,* et al. Trace element status (Se, Cu, Zn) in healthy Portuguese subjects of Lisbon population: a reference study. Biol Trace Elem Res 2004; 101(1): 1−17.
29. *Neggers YH, Bindon JR, Dressler WW.* The relationship between zinc and copper status and lipid levels in African-Americans. Biol Trace Elem Res 2001; 79(1): 1−13.
30. *Ghayour-Mobarhan M, Shapouri-Moghaddam A, Azimi-Nezhad M, Esmaeili H, Parizadeh SM, Safarian M,* Ferns GA. The relationship between established coronary risk factors and serum copper and zinc concentrations in a large Persian cohort. J Trace Elem Med Biol 2009; 23(3): 167−75.

Received on April 17, 2014.
Revised on September 19, 2014.
Accepted on September 22, 2014.
Online First November, 2014.